Original article

The protective effect of processed cheese against hyperlipidemia in rats

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Abstract – The purpose of this study was to examine the effects of intake of processed cheeses containing different types of fats on plasma lipid profile and lipid peroxidation using hypercholesterolemic rats as an experimental model. This study included three types of processed cheeses: the first two contained vegetable oils and the third contained milk fat only. Five groups of rats (8 animals each) were fed balanced diet (normal), hypercholesterolemic diet (control), and hypercholesterolemic diet containing one of the tested processed cheeses, respectively, for 8 weeks. The body weight and the food intake were recorded and the gain in body weight and food efficiency ratio were also calculated. Blood analysis was carried out at the end of the experiment for total lipids and cholesterol, low density lipoprotein, high density lipoprotein (HDL), triglycerides, and plasma malondialdehyde. Feeding the different processed cheeses with hypercholesterolemic diet showed variable reductions in the plasma lipids, except for HDL, which was increased as compared to control. The highest and significant reduction was observed in the group that received processed cheese containing milk fat only. Also, this group showed significant reduction in lipid peroxidation. These results suggest that consumption of processed cheeses may improve the markers of cardiovascular diseases, particularly processed cheeses containing milk fat only. Further studies on human subjects are needed to verify the present results and to elucidate the possible mechanisms behind the differential effects on serum cholesterol of cheeses containing different types of fats.

processed cheese / rat / hyperlipidemia / hypercholesterolemia / lipid profile / conjugated linoleic acid

摘要 – 再制干酪对高血脂症大鼠的保护作用。 本研究探讨了高血脂症大鼠实验模型饲喂不同类型脂肪的再制干酪对血脂和脂质过氧化的影响。 使用三种类型的再制干酪，前两种使用植物油，第三种只使用乳脂肪。 五组均衡饮食 (正常组) 的大鼠 (每组 8 只) 和高血脂症饮食 (对照组)，而高血脂症饮食组分别饲喂 测试干酪 8 周。 记录体重和食物的摄入量，并对体重和食物的效率比进行了计算。 实验结束后大鼠的血液分析包括总血脂、胆固醇、低密度脂蛋白、高密度脂蛋白、甘油三酯 (Tg) 和丙二醛 (MDA)。 结果表明，饲喂不同再制干酪的高血脂症大鼠血脂出现不同程度降低，但高密度脂蛋白增加；而饲喂只含乳脂肪干酪的大鼠组血脂和高密度脂蛋白降低的最显著，同时，还能显著降低脂质过氧化反应。 因此，食用再制干酪特别是只含有乳脂肪的再制干酪可改善心血管疾病。 有必要进行人体实验以验证本实验结果，并探讨含有不同类型脂肪的干酪对血清胆固醇可能的影响机制。

再制干酪 / 大鼠 / 高脂血症 / 高胆固醇血症 / 脂质特性 / 共轭亚油酸

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Résumé – Effet protecteur du fromage fondu contre l'hyperlipidémie chez le rat. Le but de cette étude était d'examiner les effets de la consommation de fromages fondus contenant différents types de matière grasse sur le profil lipidique du plasma et la peroxydation des lipides sur le rat hypercholestérolémique comme modèle experimental. L'étude incluait trois types de fromages fondus ; les deux premiers contenaient des huiles végétales et le troisième uniquement de la matière grasse laitière. Cinq groupes de rats (de 8 animaux chacun) ont bénéficié pendant 8 semaines d'un régime alimentaire équilibré (normal), hypercholestérolémique (contrôle) ou hypercholestérolémique contenant un des fromages fondus testés. Le poids et la prise alimentaire ont été relevés et le gain de poids et le ratio d'efficacité alimentaire ont été calculés. À la fin de l'expérimentation, une analyse du sang a été réalisée pour les lipides totaux et le cholestérol, LDL et HDL, les triglycérides et le malondialdéhyde du plasma. L'apport des différents fromages fondus à un régime hypercholestérolémique a montré des réductions variables des lipides du plasma, à l'exception des HDL qui augmentaient comparativement au contrôle. La réduction significative la plus élevée était observée dans le groupe qui recevait le fromage fondu contenant uniquement la matière grasse laitière. Ce groupe présentait également une réduction significative de la peroxydation des lipides. Ces résultats suggèrent que la consommation de fromages fondus peut améliorer les marqueurs de maladie cardiovasculaire, en particulier les fromages fondus contenant uniquement de la matière grasse laitière. Des études complémentaires sur des sujets humains sont nécessaires pour vérifier les présents résultats et pour élucider les mécanismes possibles sous-jacents aux différents effets sur le cholestérol sérique des fromages contenant différents types de matière grasse.

fromage fondu / rat / hyperlipidémie / hypercholestérolémie / profil lipidique / acide linoléique conjugué

1. INTRODUCTION

Current dietary guidelines recommend limiting the consumption of foods high in saturated fat and cholesterol to reduce the risk of cardiovascular disease (CVD) [6]. Milk fat is generally recognized to consistently raise plasma cholesterol concentration, especially in hypercholesterolemic subjects [14, 17, 30]. For this reason, consumption of high fat dairy products would be expected to be associated with CVD risk. However, results of epidemiologic surveys, animal experiments, and human dietary studies have been conflicting [27, 28, 36, 38]. This discrepancy may be ascribed to the differences in the atherogenic effects of various dairy products.

Epidemiologic evidence suggests that cheese may be less atherogenic than other high fat dairy products such as butter [3, 29]. Animal experiment [33] suggested that the fat derived from cheese could offer some significant reduction in CVD risk markers. This was apparent from the lower

total and non-HDL-Ch (high density lipoprotein cholesterol) and increased percentage of long chain ω-3 fatty acids in liver triglycerides (TG) of rats fed on cheese diet, as compared with the tallow-based diet [33]. The long chain ω-3 fatty acids are known by their protective effect on the CVD [26]. Recently, a study based on conventional and metabonomic approaches showed that the lowest atherogenicity was obtained with canola cheese diet followed by the dairy fat cheese diet, while the greatest atherogenicity was observed with the butter diet ($P < 0.05$) in hyperlipidemic hamsters [23]. Also, in the controlled dietary study on human subjects [3, 29], cheese was less cholesterol increasing than butter at equal fat content. It is possible that other constituents found in a high concentration in cheese may modulate the CVD risk of cheese. Cheeses are rich sources of dairy calcium, which has been reported to decrease fat adsorption [22] and increase fecal fat excretion [2, 4]. Also, conjugated linoleic acid (CLA), butyrate, and sphingolipids

and metabolites of bacteria involved in cheese ripening were suggested as potential factors in the beneficial effects of cheese on CVD risk [3, 16, 29, 33, 38].

Out of the different cheeses examined, the effect of processed cheese consumption on the CVD risk factors is almost unknown. This group of cheese is made from the blends of natural cheese and other dairy products (e.g. butter and skim milk powder) with emulsifying salts and several food additives and heated at high temperatures.

In Egypt, addition of saturated vegetable oils with similar melting properties to milk fat (e.g. palm, palm kernel, and coconut oils) in processed cheese manufacture is permitted and the products are covered by a separate standard [11]. Vegetable oils represented a significant part of the cheese fat in several processed cheese brands in Egypt [8]. The use of vegetable oils in the replacement of milk fat in processed cheese making may affect the atherogenic properties of the cheese. The type of fat has a more important role in determining the risk of CVD rather than the total amount of fat in the diet [14, 17].

The objective of this study was to evaluate the effect of ingestion of processed cheese containing different types of fats on the plasma lipid profile and the lipid peroxidation of hypercholesterolemic rats.

2. MATERIALS AND METHODS

2.1. Cheese

Three types of processed cheeses were purchased from the local market. Processed cheese Nos. 1 (**Veg 1**) and 2 (**Veg 2**) were labeled to contain vegetable oils, while processed cheese No. 3 (**MF**) contained only milk fat. The fatty acid composition of these cheeses has been reported in a previous study [8]. They had the following fatty acid composition (Tab. I). **Veg 1** was characterized by high C_{16}, C_{18}, and $C_{18:2}$ contents, while **Veg 2** was characterized by high C_{12} and

Table I. Fatty acid composition of processed cheeses (% of total).

Compound	Veg 1	Veg 2	MF
C_4	0.48	0.65	3.86
C_6	0.25	0.67	2.37
C_8	0.21	3.81	1.32
C_{10}	0.36	3.24	2.85
$C_{10:1}$	0.03	0.04	0.31
C_{12}	1.93	34.42	3.30
C_{14}	2.59	11.85	10.50
$C_{14:1}$	0.04	0.13	0.89
C_{15}	0.24	0.20	1.13
C_{16}	40.65	13.09	29.20
$C_{16:1}$	0.33	0.26	1.66
C_{17}	0.19	0.13	0.52
C_{18}	5.23	13.80	10.03
$trans(9+10+11)\ C_{18:1}$	0.45	5.60	3.53
$C_{18:1}$	35.43	9.83	18.63
$C_{18:2}$	9.32	0.80	1.13
$C_{18:3}$	0.19	0.06	0.83
CLA	0.18	0.15	1.31
Others	1.91	1.29	6.64
SFA[*]	52.13	81.86	65.08
$C_{12} + C_{14} + C_{16}$	45.17	59.36	43.00
MUFA[*]	36.69	15.86	25.02
PUFA[*]	9.69	1.01	3.27

From reference [8] (reprinted with permission from the Egyptian Society of Dairy Science).
[*] SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

C_{14} contents and **MF** by high contents of C_4, C_6, CLA, and $C_{18:3}$. The type and the percentage of vegetable oil in **Veg 1** and **2** were not given in the label, as it is not mandatory in the Egyptian Standard for processed cheeses and spreads containing vegetable oils [11]. As milk fat is the only known fat to contain C_4 [41], the approximate milk fat and vegetable oils in cheese fat can be calculated from its C_4 content. Accordingly, **Veg 1** and **2** contained ~ 88% and 83%, respectively, of vegetable oil/total cheese fat.

2.2. Animals

Male Albino rats of 103–110 g body weight were used in this study. Although

Table II. Composition of different experimental diets (g·100 g^{-1})[**].

Ingredients	Hypercholesterolemic diets				Balanced diet
	Veg 1	**Veg 2**	**MF**	Control	
Casein[*]	2.6	2.9	2.6	11.6	11.6
Corn oil	–	–	–	–	6.5
Palm oil	–	–	–	20	3.5
Sucrose	39.1	39.1	39.1	39.1	39.1
Starch	9.4	8.7	12.9	19.5	28.8
Salt mix	1.3	1.3	1.0	3.5	3.5
Vitamin mix	1	1	1	1	1
Fiber	–	–	–	–	1
Cholesterol	0.3	0.3	0.3	0.3	–
Cheese samples	41.4	41.7	38.1	–	–

[*] 11.6 g·100 g^{-1} casein contained 10 g protein.
[**] All diets contained 20% fat except balanced diet that contained 10% fat.

rats are not considered as the best model to study the effect of dietary lipids on serum lipoprotein concentration and metabolism, they are widely used in several studies as they generally support observations in human beings [30, 43]. The animals were kept individually in stainless steel cages in the animal house at room temperature (20 ± 2 °C) with a 12-h light-dark cycle. The institution's Guidelines for the Care and Use of Laboratory Animals were followed. Diet and water were given ad libitum.

2.3. Cholesterol

Analytical grade cholesterol was obtained from Sigma, St. Louis, USA.

2.4. Chemical analysis of processed cheese types

All the fresh cheese samples were analyzed for moisture, protein, fat, and ash using standard AOAC procedures [1]. Cheese fat was extracted [18], methyl esters of fatty acids were prepared [19] and analyzed using gas liquid chromatograph

(GLC) (HP 6890 Series (Agilent Technologies, Palo Alto, CA)) [8].

2.5. Preparation of processed cheese for animal feeding

Cheese samples were dried in an air oven at 40 °C and ground before the preparation of different diets.

2.6. Preparation of diets

Balanced diet (normal), hypercholesterolemic diet (control) and three hypercholesterolemic diets each containing one type of processed cheese examined were formulated as given in Table II. A high level of cholesterol (0.3%) was added to the diet in order to create a stressful dietary model similar to studies in which CLA was protective against atherosclerosis [32, 35]. The cholesterol brought in by the diet from the added cheese would be higher in the diet containing milk fat compared to **Veg 1** and **2** diets, as vegetable oils do not contain cholesterol. However, the contribution of cheese in the overall cholesterol content of hypercholesterolemic diet is considered small (< 10%

of total cholesterol (T-Ch) in the diet) and was not taken into account, as the experiment was intended to test the protective effect of cheese per se. The presence of oxidized cholesterol in the added reagent was not considered similar to all cited studies [32, 35]. In addition, oxidized cholesterol in processed cheeses was usually found only in very small contents [37].

Different diets contained 5% moisture, 10% protein, 20% fat (except the normal balanced diet that contained 10% fat), 39.1% sucrose, 1% vitamin mixture, 3.5% salt, and completed to 100% by starch. The salt mixture and the vitamin mixture were prepared according to [5, 25], respectively. Oil soluble vitamins were given orally at a dose of 0.1 mL/rat per week. The total calories were 482 kcal·100 g^{-1} of experimental diets and 392 kcal·100 g^{-1} in the balanced diet. In all diets, fat energy represented 37.3% of total dietary energy.

2.7. Design of experimental study

Forty rats were divided into five groups (eight rats each). Groups 1–3 received hypercholesterolemic diets containing one of the different processed cheeses, group 4 received hypercholesterolemic diet (control), and group 5 was fed on balanced diet (normal) for 8 weeks. The experimental period of 8 weeks has been chosen in line with the previous studies [12, 20, 33]. During the experiment, body weight and food intake were recorded weekly. At the end of the experiment, the total food intake, body weight gain, and food efficiency ratio were calculated. Blood samples were collected from all the rats at the end of the study, after an overnight fast. Plasma was analyzed colorimetrically using specific kits (Stanbio Laboratory, Boerne, TX, USA) for total lipids [39], T-Ch [42], HDL-Ch [7], low density lipoprotein cholesterol (LDL-Ch) [13], and TG [24]. The HDL-Ch/T-Ch ratio was calculated. Plasma malondialdehyde (MDA) was determined

Table III. Average composition of processed cheese samples.

Constituents	g·100 g^{-1} dry matter		
	Veg 1	Veg 2	MF
Protein	18.8	18.00	21.5
Fat	50.9	50.5	55.2
Ash	5.6	5.5	6.8
Carbohydrates*	24.7	26.0	16.5

* Carbohydrates were calculated by differences.

colorimetrically as a thiobarbituric acid value [34] as an indicator for lipid peroxidation.

2.8. Statistical analysis

One-way analysis of variance followed by Duncan's test was applied between the different experimental groups. In all cases, $P < 0.05$ was used as the criterion of statistical significance.

3. RESULTS

Processed cheese samples demonstrated fat/dry matter content that ranged from 50.47% in **Veg 2** to 55.22% in **MF**, while **Veg 1** showed 50.89% fat content.

The proximate composition (% per dry matter) of cheese samples is shown in Table III. **Veg 1** and **2** cheeses had nearly the same gross composition while **MF** cheese was characterized by higher protein, fat, and ash contents, and lower carbohydrate content than the other two cheeses. This can be attributed to the possible inclusion of high percentages of skim milk powder and/or whey powder in **Veg 1** and **2** as compared to **MF**. The high carbohydrate content in **Veg 1** and **2** may support this conclusion. Based on the relative butyric acid and CLA contents (Tab. I), the estimated vegetable fat/total cheese fat was about 88% and 83% for **Veg 1** and **2**,

Table IV. Nutritional parameters of different experimental groups.

Groups		Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total food intake (g)	Food intake (g·day^{-1})	Food efficiency ratio
Veg 1	Mean	108.90	174.10^b	65.30^b	574.00^b	10.30^b	0.11^b
	± SE	2.14	2.94	1.63	6.85	0.12	0.01
Veg 2	Mean	108.90	208.60^a	99.80^a	628.90^a	11.20^a	0.16^b
	± SE	1.89	2.49	2.34	7.89	0.14	0.01
MF	Mean	108.80	218.00^a	109.30^a	647.30^a	11.60^a	0.17^a
	± SE	1.59	4.99	4.31	7.07	0.13	0.01
Control	Mean	108.90	215.30^a	106.50^a	641.80^a	11.50^a	0.17^a
	± SE	2.00	3.75	3.58	6.84	0.12	0.01
Normal	Mean	108.40	219.10^a	110.80^a	670.80^a	12.00^a	0.17^a
	± SE	0.82	2.08	1.74	3.29	0.06	0.01

Data with different superscripts in the same column are significantly different ($P < 0.05$).

respectively, which suggests that smaller quantities of natural cheese were used in their formulations than in **MF** cheese.

Nutritional parameters of different experimental groups are shown in Table IV. There was no difference in the final body weight, body weight gain, and food intake, except in rats fed on hypercholesterolemic diet containing processed cheese 1, which showed a significant reduction in all nutritional parameters ($P < 0.05$). Food efficiency ratio was reduced significantly in rats fed on hypercholesterolemic diets containing processed **Veg 1** and **2** when compared with the control group. The nonsignificant changes in nutritional parameters especially food intake and final body weight in rats fed on hypercholesterolemic diet containing processed **MF** cheese probably reflect the palatability of the processed cheese used in this study, while processed **Veg 1** seems to reduce diet palatability, as shown from all determined nutritional parameters.

The control rats fed the hypercholesterolemic diet showed a significant increase in the plasma levels of total lipids (+40%, $P < 0.05$), T-Ch (+20%, $P < 0.05$), LDL-Ch (+14%, $P < 0.05$), and TG (+17%,

$P < 0.05$) (Tab. V), which was accompanied by a decrease in the HDL-Ch and the HDL-Ch/T-Ch ratios (−31% and −85%, respectively, $P < 0.05$) (Tab. V). Plasma level of MDA (Tab. V) as an indicator of lipid peroxidation was increased significantly (+61%, $P < 0.05$) in hypercholesterolemic rats when compared with normal rats fed on balanced diet.

Inclusion of processed cheeses in hypercholesterolemic diets improved variably the biochemical parameters of plasma (Tab. V), as compared to the control. Generally, **Veg 1** showed the lowest improvement in the plasma lipid profile, while **MF** showed the highest improvement in the plasma lipid profile and peroxidation in hypercholesterolemic rats. Statistical analysis showed that the **MF** was the only processed cheese that significantly improved the lipid profile and peroxidation of hypercholesterolemic rats.

4. DISCUSSION

Feeding rats a diet rich in cholesterol evoked an increase in risk factors for atherosclerosis and CVD such as dyslipidemia

Table V. Biochemical parameters of different experimental groups.

Groups		T. lipids (g·dL^{-1})	T-Ch (mg·dL^{-1})	HDL-Ch (mg·dL^{-1})	LDL-Ch (mg·dL^{-1})	HDL-Ch/ T-Ch ratio	TG (mg·dL^{-1})	MDA (nmol·mL^{-1})
Veg 1	Mean	0.52[a]	108.00[a]	32.30[a]	27.1[a]	0.30[a]	109.30[a]	8.30[a]
	± SE	0.01	2.09	0.33	0.43	0.05	2.48	0.21
	%change	−7	−3	−0.4	−2	3	−0.4	4
Veg 2	Mean	0.59[a]	106.30[a]	33.00[a]	26.7[a]	0.30[a]	104.40[a]	7.70[a]
	± SE	0.01	1.16	0.65	0.5	0.01	2.40	0.13
	%change	−6	−5	2	−4	7	−5	4
MF	Mean	0.55[b]	100.60[b]	40.00[b]	25.1[b]	0.40[b]	97.50[b]	5.90[b]
	± SE	0.01	1.18	0.53	0.33	0.01	1.42	0.25
	%change	−16	−10	23	−9	37	−11	−26
Control	Mean	0.61[a]	111.70[a]	32.50[a]	27.7[a]	0.30[a]	109.70[a]	7.90[a]
	± SE	0.01	1.95	0.54	0.44	0.01	3.04	0.19
	%change	40	20	−31	14	−43	17	61
Normal	Mean	0.44[c]	92.80[c]	47.20[c]	24.4[c]	0.51[c]	93.70[c]	4.90[c]
	± SE	0.02	1.49	0.93	0.62	0.01	1.30	0.26

Data with different superscripts in the same column are significantly different ($P < 0.05$).

(high TG, total- and LDL-Ch, and low HDL-Ch) and lipid peroxidation (increased MDA levels). This general profile elicited by an unhealthy dietary pattern (atherogenic diet) was reverted variably when different processed cheeses were included in the diet. Consumption of processed cheese-based diets reduced the total plasma lipids, T-Ch, LDL-Ch, and TG and increased the HDL-Ch/T-Ch ratio as compared to the control group receiving hypercholesterolemic diet. These effects were more pronounced in an **MF**-based diet. Several factors may be responsible for the observed differences in the protective effect of processed cheeses against hyperlipidemia in rats. Higher percentage of natural cheeses was probably used in the production of **MF** cheese compared to the other two cheeses as apparent from the low carbohydrate (mainly lactose) content in this type of cheese (Tab. II). Also, **Veg 1** and **2** were estimated to contain 88% and 83% vegetable oils in the total cheese fat, respectively. This means that the natural cheeses would account for about 20% solids in these two types of cheeses, as compared to > 50% natural cheeses in **MF** (without vegetable oils). Previous report [28, 33] showed that the consumption of natural cheeses had a beneficial effect on CVD risk markers (High TG, total- and LDL-Ch, and low HDL-Ch). Based on that the atherogenic characteristics of processed cheeses are probably related to the level of natural cheese used in the blend; the more natural cheese the less atherogenic is the obtained processed cheese. C_{12}, C_{14}, and C_{16} are considered as the most potent saturated fatty acids in raising LDL-Ch in various animal models [30]. The sum concentrations of these acids in **Veg 1** and **2** were 45.17% and 59.36%, being higher than their content in milk fat; namely 43.0% (Tab. I) which may explain the higher atherogenic effect of **Veg 1** and **2** than **MF**. The carbohydrate contents of the different diets may have no effect on the serum lipid profile. Starch and lactose are the carbohydrates found in the experimental diets. Tholstrup [38] found that the addition of lactose to cheese to match this component in milk did not affect the low LDL-Ch in subjects consuming

the reduced lipid peroxidation in the group of rats receiving this diet. Kyeong et al. [20] reported that dietary CLA reduced lipid peroxidation by increasing oxidative stability in rats, being in support with the present finding. It is suggested that other markers of oxidative stress (e.g. 4-hydroxyalkenals and isoprostanes) are to be performed in future studies in order to validate the effect of CLA on serum lipid peroxidation.

5. CONCLUSION

The intake of processed cheeses containing different types of fats improved variably the cardiovascular marker in hypercholesterolemic rats with normal processed cheese (containing milk fat only), which was more effective than cheeses containing vegetable oils. This study suggests that consumption of processed cheese containing milk fat may be beneficial in reducing the risk of CVDs. Further studies on human subjects are needed to verify the present results and to elucidate the possible mechanisms behind the differential effects on serum cholesterol of cheeses containing different types of fats.

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